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Meat intake, cooking-related mutagens and risk of colorectal adenoma in a sigmoidoscopy-based case-control study

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Abbreviations used in the text:

MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline); DiMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine); BP (Benzo[*a*]pyrene); PAH (Polycyclic aromatic hydrocarbon); OR (odds ratio); 95% CI (95% confidence interval); BBQ (barbecue).

Abstract

Reported habits of red meat consumption, particularly red meat that has been cooked to the degree termed 'well-done,' is a positive risk factor for colorectal cancer. Under high, pyrolytic temperatures, heterocyclic amines (HCA) and benzo[*a*]pyrene (BP) molecules can form inside and on the surface of red meat respectively. These compounds are precursors that are metabolically converted to compounds known to acts as mutagens and carcinogens in animal models, yet their role in human colorectal carcinogenesis remains to be clarified. We investigated whether intake of these compounds is associated with risk of colorectal adenoma in the context of a polyp-screening study conducted in Southern California. Using a database of individual HCAs and BP in meats of various types and subjected to specified methods and degrees of cooking, we estimated nano-gram (ng) consumption of PhIP, DiMeIQx, MeIQx and BP. We observed a 6% increased risk of large (>1cm) adenoma per 10ng/day consumption of BP [OR = 1.06 (95% CI, 1.00-1.12), p (trend) =0.04]. A major source of BP is red meat exposed to a naked flame, as occurs during the barbecuing process. Consistent with this finding an incremental increase of 10g of barbecued red meat per day was associated with a 29% increased risk of large adenoma [OR = 1.29 (95% CI, 1.02-1.63), p (trend) =0.04]. Individuals in the top quintile of barbecued red meat intake were at increased risk of large adenoma [OR = 1.90 (95% CI, 1.04-3.45)], compared with never consuming barbecued red meat. The consumption of oven-broiled red meat was inversely related to adenoma risk compared with non-consumers [OR = 0.49 (95% CI, 0.28-0.85)]. We did not identify any association with consumption of individual HCAs and colorectal adenoma risk. These results support the hypothesis that BP contributes to colorectal carcinogenesis.

Introduction

Several cohort and case-control studies have indicated that high intake of red meat may be related to colorectal neoplasia. A meta-analysis of meat consumption and colorectal cancer susceptibility found that a daily increase of 100g of total meat or red meat was associated with a 12-17% elevated risk of colorectal cancer, while a daily increase of 25g of processed meat was associated with a 25% elevated risk (1). Enhanced risk of colorectal adenoma, an established precursor lesion of colorectal cancer, has also been associated with high levels of red meat consumption in some studies (2,3) but not in others (4,5). A biological mechanism whereby meat consumption may predispose to these conditions has not been established, although several classes of compounds have been implicated. Heterocyclic amines (HCA), such as PhIP, MeIQx and DiMeIQx, and polycyclic aromatic hydrocarbons (PAH), such as BP, are both formed when meat is exposed to high, pyrolytic temperatures (6,7,8). HCAs are highly mutagenic in Ames *Salmonella* tests and induce gastrointestinal tumors when fed in high levels to rodents (9), and BP are metabolically converted to metabolites that can form DNA adducts (10). In addition to HCAs and BP, red meat increases fecal *N*-nitroso compounds in a dose-dependent manner (11); these compounds have exhibited carcinogenic activity in rodent models (12).

The carcinogenic potential of HCAs in the human colon has been the focus of intensive research in recent years. Studies that have used surrogates for HCA and BP exposure, such as type of meat consumed and cooking method, have produced results which were, in some instances, supportive of the HCA and PAH hypotheses (13,14).

To more specifically test these hypotheses, we developed a meat-cooking module and HCA/BP/mutagenic activity database that contains estimates of the quantity of individual HCA and BP compounds present in various types of meat cooked by each of the common methods to specified degrees of doneness (7,8,14). We previously used the database to analyze data from a different case-control study of colorectal adenomas, and found occurrence of polyps to be associated with both HCA intake and estimated mutagenic activity from cooked meat reportedly consumed by study participants (15).

In the sigmoidoscopy-based case-control study of distal adenomas addressed in the present analysis, we previously examined effects of crude meat intake, cooking method and degree of doneness of consumed meat on the risk of colorectal adenoma (13). In the analysis we report here, we explored effects of estimated intake of individual HCAs and BP intake, as assessed by a meat-related mutagen module and HCA/BP database among the subset of subjects who provided the information needed to estimate intake of these compounds. The assessment of exposure to specific meat-derived compounds will aid the elucidation of the mechanisms linking meat consumption to colorectal carcinogenesis.

Materials and Methods

Subjects Participants were recruited from two southern California Kaiser Permanente Medical Centers (Bellflower or Sunset) as described previously (13). In short, all underwent a flexible sigmoidoscopy during the period January 1, 1991 through August 25, 1993. Eligible subjects were English speaking, aged 50-74 and residents of Los Angeles or Orange counties. Exclusion criteria included severe gastrointestinal symptoms, invasive cancer, inflammatory bowel disease, familial polyposis, previous bowel surgery, or disability precluding an interview. Cases had a first time diagnosis of histologically confirmed adenoma. Controls were selected from participants with no history of colorectal neoplasia for whom no polyp was detected during the sigmoidoscopy, and matched to cases by gender, age (within 5 year category), date of sigmoidoscopy (within 3 month category), and center.

Of 628 eligible cases and 689 controls, 70 cases and 94 controls refused interviews and 29 cases and 32 controls could not be contacted. The overall response rate was 84% among cases and 82% among controls for the provision of baseline information. Five hundred and twenty nine cases and 562 controls were re-contacted and asked to provide more specific meat cooking and preparation information. Of these, two hundred and seventy five cases and 312 controls participated, corresponding to participation rates of 56% and 59%, respectively. Between January 17, 1995 and May 26, 1998, 261 cases and 304 controls completed a meat-cooking module. The study was approved by the Human Subjects Protection Committees of the University of California, Los Angeles, and the University of Southern California, and the Kaiser Permanente Institutional Review Board.

Baseline information At baseline, participants provided data on smoking, therapeutic drug use, physical activity, height, weight, family history of cancer and other factors during a 45-minute in-person interview. They also completed a semi-quantitative food frequency questionnaire (FFQ) that queried habitual diet in the year prior to sigmoidoscopy. Information regarding the preparation of meat in the year before sigmoidoscopy was obtained during the in-person interview. Participants provided crude information on how red meat was usually cooked [possible answers: rare (still very red); medium rare; medium; medium well-done; well-done (no pink at all)], and on the length of time that red meat was fried (cooked on a flat, heated surface, with or without added oil), oven-broiled (cooked in an oven under a heat source), baked (cooked in an oven with very little or no added liquid), barbecued on an outdoor grill (cooked on an open grate over a heat source), or prepared by another method. Participants were asked whether, in general, they ate meat that was lightly browned, medium browned or darkly browned.

Meat cooking module All participants completed an FFQ containing detailed questions on frequency of intake and cooking techniques for the following meat items: hamburgers/cheeseburgers, beef steaks, pork chops, bacon, sausage, fried chicken, chicken or turkey (including sandwiches), fried fish/fish sandwich, other fish except fried, and meat gravies. In addition, these subjects provided information regarding their usual preference for level of internal doneness and external browning of hamburgers/cheeseburgers and beef steaks, and doneness for pork chops, bacon, chicken, and sausages. Consumption of different types of gravies and of fat from fried bacon was also assessed. Based on these data, we estimated intake of HCAs, (MeIQx, DiMeIQx, and PhIP) and BP using a database developed previously (6,7,8,16). In order to estimate these values, we first estimated gram consumption of each meat item using frequency and standard portion size (small, medium or large). We also calculated gram consumption of all meats combined, of the five red meat items for which we had information describing cooking methods and degree of doneness (beef steak, hamburger/cheeseburger, pork chops, sausage and bacon), and of white meat (chicken and fish). Gram consumption was also calculated for red and white meat items cooked by a specific method (BBQ, broiling, frying) and/or to a specific degree of doneness (rare/medium; well-/very well-done). The data from the FFQ was used to determine meat consumption frequency, portion size, cooking technique, and doneness level. These data were used in conjunction with an established database of HCA and BP concentrations found for a variety of meats cooked by different methods to a range of doneness levels to estimate exposure to these meat-related mutagens.

Data analysis The present analysis was restricted to subjects with complete information from the meat cooking module for which we could estimate consumption levels of the individual HCAs and of BP (261 colorectal adenoma cases and 304 polyp-free controls). As measures of association between these factors and risk of adenoma, we calculated odds ratios (OR) and 95% confidence intervals (95% CI) using unconditional logistic regression for both continuous and

categorical data. Construction of quintiles for meat intake and intake of meat-derived mutagens was based on distributions among the controls. Covariates considered for inclusion in the multivariate models, described in detail elsewhere *(*13*)* were center; age; gender; ethnicity; total calorific intake, consumption of fruits, vegetables saturated fat and alcoholic beverages; physical activity; past and current smoking (using separate indicators); NSAID use; body mass index (BMI); and family history of colorectal cancer. In addition to age, gender, ethnicity, and energy, we included any covariates whose exclusion from the model changed the point estimates of interest by more than 10 percent. Subgroup analyses were conducted for adenomas greater than 1cm in diameter and for individuals who reported no dietary change since screening.

Results

The characteristics of the study population are presented in Table I. Overall, cases had a higher calorific intake; drank more alcohol; consumed higher quantities of red meat, BP, DiMeIQx, MeIQx and PhIP.

Table II presents distributions and associations for meat intake and colorectal adenoma risk. The distributions of BBQ and oven-broiled red meat intake were highly skewed. Among controls, 213 (70%) reported eating no barbecued red meat, and 256 (84%) reported eating no oven-broiled red meat during the previous year, so it was not possible to define five distinct quintiles for these variables. Instead, for the barbecued meat variable, all participants who reported consuming no BBQ red meat were assigned to the reference category, and those who did report consuming barbecued meat were assigned to either the highest quintile or a middle category. For the oven-broiled red meat variable, participants were assigned to either the non-consumer or the consumer category. High intake of barbecued red meat was positively associated with risk of large adenomas [OR = 1.89 (95% CI, 1.04-3.45)] (top quintile versus non-consumers). In addition, a 10g per day incremental increase in barbecued red meat consumption was associated with a 29% enhanced risk of large adenoma (CI, 1.02-1.63, P-trend, 0.04). Conversely, the consumption of oven-broiled red meat was inversely related to colorectal adenoma risk [OR = 0.49 (95% CI, 0.28-0.85)] compared with non-consumers and a 10g increase in oven-broiled red meat consumption was associated with a 50% reduction in adenoma risk [OR = 0.46 (95% CI, 0.24-0.89)].

Table III presents associations between estimated intake of cooking-related mutagens and risk of colorectal adenoma. We observed no association between disease risk and high consumption of the individual HCA compounds. However, there was an association between BP and risk of adenomas greater than 1cm in diameter with a non-significant 1.77-fold increased risk associated with the highest quintile of BP intake compared with the lowest quintile (95% CI, 0.76-4.12). Furthermore, a 10ng/day incremental increase in BP consumption was associated with a 6% increased prevalence of adenomas greater than 1cm (CI, 1.00-1.12; P-trend, 0.04).

When we restricted analyses to individuals with no reported dietary change since screening, we observed no material change in the risk estimates (data not shown).

Discussion

In this sigmoidoscopy-based study of 261 colorectal adenoma cases and 304 polyp-free controls, we found evidence for an association between barbecued red meat and BP intake and prevalence of adenomas greater than 1cm in diameter. In addition, consumption of oven-broiled red meat was negatively related to adenoma risk. There was no suggestion of an association between consumption of HCA and disease risk in this population.

A number of studies have sought to investigate the association between colorectal neoplasia and meat doneness and cooking methods. These have in general yielded

heterogeneous results that may be explained by the use of dietary questionnaires that enquired about consumption of well-done meat, but did not address methods used to cook meat. Cooking methods are now known to be important determinants of the quantities of various classes of pro-carcinogens present in meat. In an attempt to account for the effects of cooking method in estimates of HCAs and PAH in consumed meat, a FFQ was designed to include a meat-cooking module that enquired into specific meat-cooking practices and preference for well-done meat. When this FFQ was employed in an earlier case-control study of colorectal adenomas an increased risk of adenoma was found to be associated with high intake of well-done and grilled or pan-fried red meat (14). To explore the role of meat-derived mutagens such as HCAs and BP in colorectal carcinogenesis, the module was used to estimate quantities of specific HCAs and BP and from meats. When this approach was used to analyze data from the aforementioned separate study population, total mutagenic activity from cooked meat and intake of MeIQx were positively associated with colorectal adenoma risk (15).

We have now used the same method to investigate the role of meat-related mutagens in the present sigmoidoscopy-based case-control study of colorectal adenoma. It had previously been shown in this population that frequent consumption of darkly-browned red meat and fried red meat was positively associated with risk of colorectal adenoma (13). Since fried meat is a major source of HCAs whereas BP is generally formed when meat is grilled or barbecued, we anticipated that both HCA and BP would be associated with adenoma risk in this population. However, in the present study, we found only BP intake to be significantly related to disease risk. For large adenomas, there was a suggestion of an association with DiMeIQx and PhIP, but risk estimates did not attain statistical significance. Our failure to corroborate the findings of Sinha et al. may be a consequence of differences in dietary practices between the two study populations and the non-significant risks associated with DiMeIQx and PhIP suggest that we cannot reject the hypothesis that HCA exposure is a risk factor for colorectal adenoma.

One of the strengths of this investigation was that our study population had participated in a polyp-screening trial hence our control group was free of neoplastic lesions in the left-side of the colon. However, we cannot exclude the possibility that polyps went undetected in the right-side of the colon, thus attenuating our results under the assumption that HCA and BP are risk factors of both left- and right-sided adenomas. Another potential concern is that although we obtained detailed information on meat cooking practices, responses were provided retrospectively, introducing the potential for recall bias. However, this is less likely to be important for pre-cancerous polyps that are removed during sigmoidoscopy and require no further treatment. Furthermore, we observed no change in the risk estimates when analyses were restricted to those with no reported dietary change. One further limitation of the FFQ is that participants reported only habitual diet from the past year. This fails to account for dietary patterns in the past, which may be more relevant for the initiating stages of carcinogenesis. Although we obtained detailed cooking practice data in this study, the participation rate in the present study was lower than in the original study of meat and colorectal adenoma risk conducted in this population, reducing statistical power and raising questions regarding selection bias.

Finally, we also must consider the limitations of the database for this study. One caveat to the use of the database is the fact that it is based on a limited number of cooking assays and is based on foods collected from a limited geographical area. In addition, the meat mutagen values were obtained from a limited number of meat samples (6,7,8). Furthermore, the estimated mutagen intakes for each individual were derived from self-reported doneness levels which may have led to some degree of misclassification.

Our observation that high intake of both barbecued red meat and BP is associated with colorectal adenoma risk is interesting considering biological evidence from animal models which relates BP exposure to tumors of amongst others, the stomach and intestine (17). An association between barbecued or grilled meats and tumors of the gastro-intestinal tract has been reported by several studies (18,19,20) and PAH-DNA adducts have been detected at high levels in leukocytes of humans exposed to char-broiled meat (21).

We observed a statistically significant inverse association between oven-broiled red meat intake and adenoma risk. Although an inverse association was unexpected, this finding supports, to some extent, the notion that it is the way that meat is cooked, rather than meat *per se* which contributes to colorectal neoplasia risk since oven-broiling meat would not be expected to produce either BP or HCAs. This does not account for the observed inverse relation and may reflect some other protective factor unaccounted for in this study.

The conversion of consumed HCAs and BP to mutagenic metabolites of these compounds appears to depend upon numerous enzymes, some of which are polymorphic and may represent important covariates not addressed in this study or the ones to which we are comparing these results. Future studies in which individual variation in the metabolism of these compounds is measured may provide further insight into the meat-colorectal neoplasia association, and identify sub-groups of the population who may be susceptible to meat-derived mutagens. For example, it was recently shown that the predicted high activity phenotype of Microsomal Epoxide Hydrolase (an enzyme on the BP metabolic pathway) was positively associated with colorectal adenoma risk among consumers of well-done meat (22). The investigation of genetic variants in enzymes that govern the metabolism of HCAs and BP is currently underway in this study population.

In conclusion, using a detailed meat-mutagen database to assess the relation between various meat-derived mutagens and risk of colorectal adenoma, we found evidence for a positive association between disease risk and intake of BP and consumption of barbecued red meat. These findings may provide some insight into the apparent association between red meat consumption and colorectal neoplasia, but they also underscore the importance of obtaining detailed information on meat-cooking practices in future studies of colorectal adenoma and cancer.

References

1. Sandhu, M.S., White, I.R. and McPherson, K. (2001) Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analysis. *Cancer Epidemiol Biomarkers Prev*, May (10):439-46
2. Neugut, A.L., Garbowski, G.C., Lee, W.C., Murray, T., Nieves, J.W., Forde, K.A., Treat, M.R., Waye, J.D., Fenoglio-Preiser, C. (1993) Dietary risk factors for the incidence and recurrence of colorectal adenomatous polyps. A case control study. *Ann Intern Med*, 118(2): 91-95.
3. Sandler, R.S., Lyles, C.M., Peipins, L.A., McAuliffe, C.A., Woosley, J.T., Kupper, L.L. (1993) Diet and risk of colorectal adenomas: macronutrients, cholesterol and fiber. *J Natl Cancer Inst*, 85(11):884-91.
4. Macquart-Moulin, G., Riboli, E., Cornee, J., Charnay, B., Berthezene, P., Day, N. (1986) Case-control study on colorectal cancer and diet in Marseilles. *Int J Cancer* 38 (2): 183-91.
5. Giovannucci, E., Stampfer, M.J., Colditz, G., Rimm, E.B., Willett, W.C. (1992) Relationship of diet to risk of colorectal adenoma in men. *J Natl Cancer Inst* 84 (2):91-8.
6. Sinha R, Rothman N, Brown ED, Salmon CP, Knize MG, Swanson CA, et al. High concentrations of the carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhiP) occur in chicken but are dependent on the cooking method. (1995) *Cancer Res*:55:4516-4519.
7. Sinha R, Knize MG, Salmon CP, Brown ED, Rhodes D, Felton JS, Levander OA, Rothman N. Heterocyclic amine content of pork products cooked by different methods and to varying degrees of doneness. (1998) *Food and Chemical Toxicology*: 36:289-297.
8. Sinha R, Rothman N, Salmon CP, Knize MG, Brown ED, Swanson CA, Rhodes D, Rossi S, Felton JS, Levander OA. Heterocyclic amine content in beef cooked by different methods to varying degrees of doneness and gravy made from meat drippings. (1998) *Food and Chemical Toxicology*:36:279-287
9. Ito N, Hasegawa R, Sano M, Tamano S, Esumi H, Takayama S, Sugimura T. (1991) A new colon and mammary carcinogen in cooked food, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). *Carcinogenesis*:12(8):1503-6.
10. Gelboin,H. (1980) Benzo(a)pyrene metabolism, activation and carcinogenesis: role of regulation of mixed-function oxidases and related enzymes. *Physiol. Rev.*, 60, 1107–1165.
11. Hughes R, Cross A.J, Pollock A.R, Bingham S.A. Dose-dependent effect of dietary meat on endogenous colonic N-nitrosation. (2001). *Carcinogenesis:* 22(1):199-202.
12. Mirvish, S.S. Role of N-nitroso compounds (NOC) and N-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC (1995). *Cancer Lett;* 93(1):17-48.
13. Probst-Hensch NM, Sinha R, Longnecker MP, Witte JS, Ingles SA, Frankl HD, Lee ER, Haile RW. (1997) Meat preparation and colorectal adenomas in a large sigmoidoscopy-based case-control study in California (United States). *Cancer Causes Control*: 8(2):175-83.
14. Sinha R, Chow W H, Kulldorff M, Denobile J, Butler J, Garcia-Closas M, Weil R, Hoover R and Rothman N. Well-done, grilled red meat increases the risk of colorectal adenomas. (1999) *Cancer Res*: 59(17):4320-4.

15. Sinha R, Kuldorff M, Chow W, Denobile J, Rothman N. Dietary intake of heterocyclic amines, meat-derived mutagenic activity and risk of colorectal adenomas. (2001). *Cancer Epidemiology Biomarkers and Prevention*: 10(5): 559-62.
16. Sinha R, Rothman N. Exposure assessment of heterocyclic amines (HCAs) in epidemiologic studies (1997) *Mutation Res*:376:195-202.
17. Rigdon, R. H. and J. Neal (1966). "Gastric carcinomas and pulmonary adenomas in mice fed benzo (a) pyrene." *Tex Rep Biol Med* 24(2): 195-207.
18. Peters, R. K., D. H. Garabrant, et al. (1989). "A case-control study of occupational and dietary factors in colorectal cancer in young men by subsite." *Cancer Res* 49(19): 5459-68.
19. Gerhardsson de Verdier, M., U. Hagman, et al. (1991). "Meat, cooking methods and colorectal cancer: a case-referent study in Stockholm." *Int J Cancer* 49(4): 520-5.
20. Muscat, J. E. and E. L. Wynder (1994). "The consumption of well-done red meat and the risk of colorectal cancer." *Am J Public Health* 84(5): 856-8.
21. Rothman, N., M. C. Poirier, et al. (1993). "Association of PAH-DNA adducts in peripheral white blood cells with dietary exposure to polyaromatic hydrocarbons." *Environ Health Perspect* 99: 265-7.
22. Cortessis V, Siegmund K, Chen Q, Zhou N, Diep A, Frankl H, Lee E, Zhu QS, Haile R, Levy D. (2001) A case-control study of microsomal epoxide hydrolase, smoking, meat consumption, glutathione S-transferase M3, and risk of colorectal adenomas. *Cancer Res*:61(6):2381-5.

Table I: Study population characteristics

Variable[a]	Cases	Controls	P[b]
N	261	304	
Mean age (years)	61.4 ± 0.64	61.7 ± 0.38	0.64
Female (%)	36.8	33.9	
Ethnicity (%)			
Caucasian	55.9	52.9	
African-American	15.3	16.8	
Hispanic	17.4	18.0	
Asian/Pacific Islander	9.6	10.2	
Regular NSAID use (%)	28.0	32.2	
Smoking history (%)			
Never	34.1	43.3	
Past	47.5	48.0	
Current	18.4	8.5	
BMI (kg/M^2)	27.6 ± 0.28	27.0 ± 0.26	0.08
Physical activity[c]	6.3 ± 1.03	6.7 ± 0.75	0.74
Energy Intake (kcal/day)	2011 ± 50.5	1954 ± 48.0	0.42
Fruit and vegetable intake (portions/day)	5.5 ± 0.20	6.3 ± 0.21	0.003
Alcohol intake (drinks/week)	5.1 ± 0.56	4.4 ± 0.48	0.30
Red meat intake (g/day)	18.1 ± 1.24	17.1 ± 1.08	0.56
White meat intake (g/day)	27.4 ± 1.53	28.4 ± 1.48	0.64
Benzo[*a*]pyrene intake (ng/day)	24.2 ± 3.21	18.0 ± 1.89	0.08
DiMeIQx intake (ng/day)	2.1 ± 0.24	1.8 ± 0.18	0.33
MeIQx intake (ng/day)	27.6 ± 2.18	24.3 ± 1.87	0.25
PhIP intake (ng/day)	101.2 ± 11.23	93.7 ± 8.49	0.59

[a] Values are means ± standard errors or % if indicated in parentheses.

[b] P-values derived from the Wilcoxon-Mann-Whitney non-parametric rank sum test.

[c] Units for physical activity are total MET (metabolic equivalent tasks-defined as the caloric need per kilogram of body weight per hour of activity divided by the caloric need per kilogram of body weight per hour at rest.) per hour per week. Includes only subjects engaging in at least 3 hours of vigorous activity per week

Table II: Adjusted odds ratios for the association between meat intake and colorectal adenoma risk

Meat intake[1]	**Total Adenomas**			**Large (>1cm) Adenomas**		
Total meat	*N*[4] **(case/control)**	**OR**	**95% CI**	*N*[4] **(case/control)**	**OR**	**95% CI**
0-16.5	49/60	1.00 (ref)	-	12/60	1.00 (ref)	-
16.6-33.7	64/61	1.18	0.68-2.02	19/61	1.5	0.6-3.4
33.9-48.0	40/66	0.67	0.38-1.19	11/66	0.7	0.3-1.8
48.1-69.7	62/56	1.22	0.70-2.10	21/56	1.6	0.7-3.7
69.9-207.1	46/61	0.80	0.45-1.42	17/61	1.1	0.5-2.6
P-trend[3]		0.55			0.6	
Red meat						
0-1.8	49/62	1.00 (ref)	-	16/62	1.00 (ref)	-
2.3-7.6	45/58	0.87	0.50-1.53	12/58	0.63	0.26-1.51
7.7-15.1	59/62	0.99	0.57-1.72	14/62	0.59	0.25-1.40
15.3-28.1	49/61	0.80	0.45-1.40	16/61	0.73	0.32-1.67
28.2-127.3	59/61	0.88	0.50-1.55	22/61	0.85	0.38-1.90
P-trend[3]		0.52			0.57	
White meat						
0-8.0	66/70	1.00 (ref)	-	21/70	1.00 (ref)	-
8.5-13.7	44/59	0.80	0.47-1.36	12/59	0.71	0.31-1.63
14.1-27.4	58/66	0.94	0.56-1.56	16/66	0.83	0.39-1.78
27.9-48.6	60/68	0.96	0.58-1.60	20/68	0.97	0.46-2.02
51.4-198.0	33/41	0.89	0.49-1.61	11/41	0.80	0.33-1.93
P-trend[3]		0.80			0.88	
Well/very well done meat						
0	71/89	1.00 (ref)	-	23/89	1.00 (ref)	-
0.5-2.3	27/32	0.86	0.46-1.62	6/32	0.50	0.18-1.41
2.8-7.6	52/61	0.86	0.52-1.45	15/61	0.58	0.26-1.31
7.7-15.4	56/61	0.97	0.58-1.62	17/61	0.71	0.33-1.53
15.7-99.1	55/61	0.84	0.50-1.40	19/61	0.79	0.38-1.68
P-trend[3]		0.55			0.88	
BBQ red meat						
0	168/213	1.00 (ref)	-	46/213	1.00 (ref)	-
1.5-6.6	29/29	1.36	0.76-2.41	9/29	1.58	0.67-3.71
7.1-94.3	64/62	1.27	0.83-1.95	25/62	1.89	1.04-3.45
P-trend[3]		0.29			0.04	
Fried red meat						
0	89/105	1.00 (ref)	-	28/105	1.00 (ref)	-
0.5-1.3	17/15	1.00	0.5-2.2	4/15	0.55	0.15-2.03
1.8-6.6	46/62	0.73	0.4-1.2	13/62	0.53	0.24-1.17
6.8-14.5	58/62	0.90	0.6-1.5	18/62	0.69	0.33-1.47
14.6-100.1	51/60	0.82	0.5-1.4	17/60	0.72	0.34-1.55
P-trend[3]		0.78			0.91	
Oven-broiled red meat						
0	237/256	1.00 (ref)	-	76/256	1.00 (ref)	-
0.3-35.0	24/48	0.49	0.28-0.85	4/48	0.22	0.07-0.67
P-trend[3]		0.02			0.15	

[1]Grams per day

[2]Adjusted for age, gender, energy, center, fruit and vegetable intake, smoking status and BMI

[3]P-trend derived from the continuous data

[4]Numbers of controls within quintiles vary in some cases due to an excess of participants reporting non-and low consumption of certain meat-types but more equal distribution in the upper quintiles.

Table III: Adjusted odds ratios for the association between meat-related mutagens and colorectal adenoma risk

Meat-related mutagen	Total Adenomas			Large (>1cm) Adenomas		
	N[3]	OR	95% CI	*N*[3]	OR	95% CI
Benzo[a]pyrene (per 10 ng/day)						
0-0.29	55/60	1.00 (ref)	-	11/60	1.00 (ref)	-
0.3-1.37	48/61	0.80	0.46-1.39	14/61	1.08	0.43-2.68
1.4-4.8	50/61	0.76	0.44-1.31	17/61	1.11	0.46-2.67
4.9-31.4	40/61	0.64	0.36-1.14	13/61	0.99	0.39-2.48
31.5-515.2	68/61	1.03	0.60-1.75	25/61	1.77	0.76-4.12
P-trend[2]		0.22			0.04	
DiMeIQx (per 10 ng/day)						
0-0.01	64/88	1.00 (ref)	-	14/88	1.00 (ref)	-
0.02-0.29	30/33	1.09	0.59-2.02	8/33	1.41	0.52-3.80
0.3-1.19	54/61	1.06	0.63-1.78	23/61	1.98	0.88-4.43
1.2-2.58	52/61	0.93	0.55-1.56	17/61	1.28	0.54-3.00
2.6-42.7	61/61	1.15	0.69-1.91	18/61	1.46	0.65-3.30
P-trend[2]		0.41			0.93	
MeIQx (per 10 ng/day)						
0-2.42	51/60	1.00 (ref)	-	14/60	1.00 (ref)	
2.48-8.4	39/61	0.73	0.4-1.3	12/61	0.80	0.33-1.96
8.5-19.08	50/61	0.88	0.5-1.5	11/61	0.62	0.25-1.54
19.1-39.9	63/61	1.03	0.6-1.8	25/61	1.35	0.59-3.08
40.5-265.5	58/61	0.89	0.52-1.55	18/61	0.89	0.38-2.06
P-trend[2]		0.63			0.99	
PhIP (per 10 ng/day)						
0-0.2	50/62	1.00 (ref)	-	11/62	1.00 (ref)	-
0.3-17.8	53/59	1.09	0.63-1.87	14/59	1.27	0.52-3.10
17.9-66.1	54/61	0.95	0.55-1.64	21/61	1.59	0.68-3.71
66.5-150.9	49/61	0.85	0.49-1.47	19/61	1.46	0.62-3.42
151.8-1846.8	55/61	1.01	0.58-1.73	15/61	1.09	0.45-2.65
P-trend[2]		0.77			0.53	

[1]Adjusted for age, gender, energy, center, fruit and vegetable intake, smoking status and BMI

[2]P-trend derived from the continuous data

[3]Numbers of controls within quintiles vary in some cases due to an excess of participants reporting non-and low consumption of certain meat-types but more equal distribution in the upper quintiles.